Studies of avian malaria and Brazil in the international scientific context (1907-1945)

SÁ, Magali Romero. Studies of avian malaria and Brazil in the international scientific context (1907-1945). *História, Ciências, Saúde – Manguinhos,* Rio de Janeiro, v.18, n.2, abr.-jun. 2011. Available at: http://www.scielo.br.

Abstract

The article explores Brazilian investigators' contributions to research on the protozoan causative agent of malaria. Focusing on the work of Henrique Aragão and Wladimir Lobato Paraense, it underscores the importance of avian malaria in elucidating human malaria and treatment options, and also examines the network of scientific relations forged by these researchers, their shared research agendas, exchange of information with other researchers, and role within the international context of scientific discoveries.

Keywords: bird malaria; Manguinhos; international relations; scientific discoveries; Brazil.

Magali Romero Sá

Researcher and professor at the
Casa de Oswaldo Cruz/Fundação Oswaldo Cruz.

Casa de Oswaldo Cruz/Fiocruz
Avenida Brasil, 4036/405
21040-361 – Rio de Janeiro – Brazil
magali@coc.fiocruz.br

Received for publication in August 2010.
Approved for publication in December 2010.

Translated by Diane Grosklaus Whitty.

"Aragão has successfully transmitted the pigeon halteridium by means of *Lynchia*, observing development within the pigeon's lung. The second issue of *Brazil-Medico* will publish a preliminary note" (Cruz, 15 abr. 1907).[1] With this excited commentary, Oswaldo Cruz informed Adolpho Lutz of the important feat achieved by Henrique de Beaurepaire Rohan Aragão, a young physician from the Manguinhos Serum Therapy Institute (Instituto Soroterápico de Manguinhos). Oswaldo Cruz indeed had reason to celebrate. Aragão's finding was one of the most valuable discoveries made at Manguinhos during that era, and it had an enormous impact in Brazil and in European centers of tropical medicine. His research into the protozoan *Haemoproteus* and its asexual development in the cells of the pigeon's pulmonary *endothelium* uncovered an important, previously unknown stage in the life cycle of the parasite. It had been believed that asexual development of the protozoan *Haemoproteus columbae* took place inside the bird's red blood cells while sexual reproduction occurred inside the Diptera insect. Aragão demonstrated that development commenced in the bird's pulmonary endothelium by schizogony, thus showing for the first time that a hemoparasite could reproduce within tissue cells.

In 1943, another researcher at Manguinhos, Wladimir Lobato Paraense, was working with the protozoan *Plasmodium gallinaceum*, a parasite of *Gallus gallus,* the species from which the domestic hen developed (*G. gallus domesticus*). This young physician from the state of Pará made major advances in our understanding of the development of the exoerythrocytic cycle of this *Plasmodium* species. His work was published in *Memórias do Instituto Oswaldo Cruz* during World War II and went overlooked by the international scientific community until 1945, when British protozoologist Charles Morley Wenyon referred to Lobato Paraense's research as one of the most successful attempts to shed light on the exoerythrocytic cycle of the malaria parasite.

The significant research that Aragão and Lobato conducted in Brazil was part of a worldwide effort to understand the life cycle of the human malaria parasite, its intermediary hosts, and disease treatment. This article explores the contributions of both Brazilian researchers within the context of international science, with a focus on the network of relationships forged during the research process and on the exchange of information with other researchers.

Protozoans, erythrocytes, and insects: similarities between human and avian malaria

Experimental research on avian hematozoa attracted greater interest after it was discovered that these parasites resemble those of human malaria. Such studies gained momentum following the notable work of Russian physiologist and protistologist Vassily Danilewsky (1852-1939), professor at the University of Kharkiv, Ukraine. In 1885, five years after the malaria *Plasmodium* had been discovered by Charles Louis Alphonse Laveran (1845-1922), Danilewsky found intraerythrocytic parasites in the blood of amphibians, birds, and reptiles and described in detail the morphology of the various forms he observed (Danilewsky, 1886).[2] He noted that parasites of birds differed from the parasites of cold-blooded animals and were quite similar to the malaria parasites described by Laveran, Ettore Marchiafava (1847-1935), and Angelo Celli (1857-1914).[3] Struck by this similarity,

Danilewsky posed the following question: were the parasites he described identical to those of human malaria? Inspired by this challenge, he conducted many anatomical and pathological investigations on infected birds and showed that the disease was accompanied by acute anemia, enlargement of the liver and spleen, and an accumulation of pigment. During his ecological research, he observed that infection of the birds and the presence of parasites in their blood were related to the seasons of the year; he concluded that intracellular blood parasites in birds were prevalent during warm seasons and that there was a correlation between parasitemia and ambient temperature. The Russian researcher was responsible for formulating the main characters of Haemosporidia[4] and for identifying the three main genera of intraerythrocytic parasites in birds (currently known as *Plasmodium*, *Haemoproteus,* and *Leucocytozoon*; Cox, 2010). In 1888, he published a monograph on bird Haemosporidia; translated from Russian to French the following year, it sparked keen interest among scientists (Valkiunas, 2005, p.10).

Laveran was one of those who took an interest in Danilewsky's results; in a paper published in 1891, he urged physicians to enter the domain of naturalists and to research birds. For him, the parasites described by the Russian protistologist were of special interest because of their great resemblance to the parasites of human malaria. Laveran believed that if the murky questions still surrounding the latter parasites were to be solved, a different path had to be taken, entailing the study of analogous parasites in a variety of animals (Laveran, 1891, cited by Slater, 2005, p.262).

Countless studies on the morphology, taxonomy, and development of protozoan parasites of birds were published starting in 1890, leading to the establishment of new genera and species. The Italian researchers who had been devoting themselves doggedly to the study of malaria turned immediately to avian malaria. From 1889 to 1892, Giovanni Battista Grassi (1854-1925) and Raimondo Feletti (1887-1927) published a number of articles on the topic. They established the genus *Haemamoeba*, encompassing the parasites of avian and human malarias, and also described the species *Haemamoeba relicta* and *Haemamoeba subpraecox* for birds and *Haemamoeba vivax* and *Laverania malariae* for humans.[5]

In 1890, German bacteriologist Walther Krause (1864-1943) established the genus *Haemoproteus* for crescent-shaped intracellular parasites previously described by Danilewsky and also described three new species within this genus: *H. danilewskii, H. columbae*, and *H. passeris*. In 1891, the Italians Angelo Celli (1861-1945) and Francesco Sanfelice tried to correlate the parasites found in birds with those of human malaria based on their development cycle; they described two new species of bird Haemosporidia: *H. alaudae* and *H. noctuae*. Celli and Sanfelice were the first to infect birds through the sub-inoculation of blood infected with avian malaria parasites. This accomplishment opened the doors to a number of laboratory research possibilities with this parasite, since the malaria *Plasmodium* could not be cultured *in vitro*.[6]

In 1891, another contribution to our understanding of the life cycle of these parasites came about by chance: while mixing the substance eosin with an oxidized solution of methylene blue, Russian scientist Dimitri Leonidovitch Romanowsky obtained a dye that distinguished the nucleus of the parasite by staining it red while staining the cytoplasm

blue, thereby highlighting cell structures.[7] This discovery also afforded better characterization and differentiation of parasites in terms of genus and species; classification of Haemosporidia was chaotic at that time, with room for contradictions.

The first scientist to point out the taxonomic discrepancies within this group of protozoans was Frenchman Alphonse Labbé. He defended his doctoral dissertation on endoglobular parasites of the blood of vertebrates in 1894; although he called attention to taxonomic incongruences, he himself included two new genera of bird parasites, *Halteridium* and *Proteosoma*, which were actually synonymous with the already established *Haemoproteus* and *Plasmodium*.[8]

Studies on human and avian malaria conducted by physicians and zoologists in laboratories around the world yielded almost simultaneous discoveries about the biological cycle of these parasites. In 1897, French physician Paul-Louis Simond (1858-1947) demonstrated the sexuated cycle of Coccidia[9] in salamanders and recognized the male sexual gamete in flagellate forms. Simond (1897) observed that these forms were found in different species of hematozoa responsible for human and avian malaria and suggested that sexual reproduction also takes place within these parasites. In the United States, William George MacCallum (1874-1944), then a student at Johns Hopkins Medical School, was studying halteridium in Corvidae in 1897 when he observed sexual reproduction in this group of protozoans *in vitro*. This was the first time that a microgamete (flagellated form) was seen to enter a macrogamete, engendering the wormlike form of the parasite now known as the ookinete. Like Simond, MacCallum imagined that the same could happen with human malaria parasites, and while examining infected human blood under the microscope shortly thereafter, he observed gametocytes shooting out flagella. MacCallum's pertinent discovery reinforced application of the avian model of the disease in the endeavor to understand the biology of the human parasite (MacCallum, 1897, 1898).

It had thus been established that there was a sexual phase in the parasitic cycle of Haemosporidia, but it remained to be established at just what moment it took place within the life cycle and how the parasite was transmitted to humans and vertebrates. Even before MacCallum's discovery, British physician Patrick Manson (1844-1922) had pointed to the process of exflagellation in malaria parasites. The emergence of flagella after contaminated blood had been examined under the microscope for some time was a sign that the parasites must continue their life cycle outside the human body (Scott, 1942). Attention then turned to blood-sucking Diptera, a hypothesis that had already been put forward by Laveran and Italian researchers, among others. The hypothesis that a mosquito was the transmitter of malaria was strengthened by Manson, who in 1877-1878 discovered the role played by *Culex* in the transmission of filariasis, and by Theobald Smith (1859-1934) and Fred L. Kilborne (1858-1936), who in 1893 demonstrated the role of ticks in the transmission of the protozoan *Pyrosoma bigeminum* (*Babesia bigemina*), the cause of Texas cattle fever. Lastly, in 1898, British physician Ronald Ross established the life cycle of the parasite of avian malaria, *Proteosoma relictum* (*Plasmodium relictum*), in *Culex*. Ross ascertained that the sexual phase of the parasite takes place in the mosquito's midgut, and he proved transmission by bite. The next year, the Italians Grassi, Bignami, and Bastinelli successfully completed the life cycle of the human malaria parasite in the mosquito of the genus

Anopheles (Cox, 2010).[10] Ross's discovery using birds confirmed that this animal model was appropriate in endeavors to elucidate human malaria.

In 1904, German researcher Fritz Schaudinn (1871-1906), then one of the world's most eminent protozoologists[11], published a study on the life cycle of a hemoparasite of the owl *Athene noctua*, which he called *Trypanosoma noctuae*. Observing the life cycle of this parasite, Schaudinn detected an alternation of sexual and nonsexual generation in the *Haemoproteus* and *Trypanosoma* forms and gave a detailed description of the sexual cycle in the mosquito *Culex pipiens*. For the German researcher, the owl *Halteridium* (*Haemoproteus*) represented merely one stage in the complicated life story of the *Trypanosoma* rather than two distinct genera, as then believed.

In 1891, the species *H. noctuae* had already been described and established by Italians Celli and Sanfelice in the same species of owl later studied by Schaudinn. The latter had allegedly clarified a still problematic question surrounding the developmental phases of hemoparasites in vertebrates, animals in which all phases of asexual production had been observed. For Schaudinn, the latter only took place inside of erythrocytes and in the blood plasma of vertebrates, where trypanosomic forms reproduced through longitudinal fission.

The alternation in forms of generation defended by Schaudinn was soon confirmed by French researchers Edmond and Étienne Sergent, that is, in 1904. But that same year, Americans Frederick George Novy (1864-1957) and Ward J. MacNeal (1881-1946) took issue with the German parasitologist, demonstrating in the blood agar[12] medium they devised that only *Trypanosoma* developed, while no alternation in the generation or growth of *Haemoproteus* parasites was observed. For Novy and MacNeal, the birds Schaudinn had used were infected by two different parasites. Despite this disagreement, the German protozoologist's work continued to receive the support of other scientists around the world, although many questions remained unanswered. Some years later, in 1907, a young researcher at the Oswaldo Cruz Institute, Henrique de Beaurepaire Rohan Aragão (1879-1955), published a paper on the life cycle of the species *H. columbae*. In addition to uncovering an unknown stage of the life cycle of the parasite (exoerythrocytic phase), Aragão confirmed Novy and MacNeal's hypothesis about the non-existence of alternation in the generation of Haemosporidia.

Studies of bird hematozoa in Brazil

When he began researching bird hematozoa, Henrique Aragão's main collocutor was physician and parasitologist Adolpho Lutz (1855-1940), then director of the São Paulo Bacteriological Institute (Instituto Bacteriológico de São Paulo).[13] Lutz was the first Brazilian researcher to turn his attention to protozoans of interest to medicine and zoology. In 1889, while still practicing medicine in Limeira, a town in rural São Paulo state, he published a paper on Myxosporidia of the gall bladder of Brazilian Batrachia. Impressed by an article published by Danilewsky some years earlier, Lutz began investigating the blood of amphibians, reptiles, and birds in search of hematozoa. In reports from the Bacteriological Institute, he described his research on human malaria with vertebrates and invertebrates

(insects), in which he observed different sporozoan protozoans[14] and their life cycles, thereby contributing, even if only indirectly, to an understanding of parasitic diseases in Brazil. Lutz focused most of his attention on birds.

From 1893 to 1907, Lutz and his team examined some twenty orders of birds and found hematozoa in nine. Following Labbé's classification, they identified *Proteosoma, Halteridium* and *Leucocytozoon* in wild and urban birds such as pigeons, crown sparrows (*tico-tico*), rails (*saracura*), crows (*gralhas*), owls, hawks, *jabirus*, *socós*, *seriemas*, tinamous (*inambu)*, *macucos*, capoeira partridges (*urus*), *jacus,* and so on, which Lutz and his collaborators used to study the development of parasites. Their research findings were released in the reports of the Bacteriological Institute and also in a communiqué at the Sixth Brazilian Congress of Medicine, published in 1907 in French and Portuguese (Lutz, Meyer, cited by Benchimol, Sá, 2005, p.889).

The young physicians at Manguinhos followed in Lutz's steps, with the malaria parasite and its insect vectors on the institute's agenda of laboratory research and preventive initiatives (see Benchimol, Silva, 2008). When Schauddin published his work in 1903, it had a major impact in scientific circles, including Brazil. It was the first time that the entire life cycle of parasites of the genus *Haemoproteus* had been uncovered in vertebrate and invertebrate hosts (*C. pipiens*). Yet despite Schaudinn's credibility, doubts were raised about his interpretation, as mentioned earlier.

Henrique Aragão joined Manguinhos in 1903, while still a medical student; he was presented to Oswaldo Cruz by Doctor Waldemar Schiller, his friend and colleague and a frequent face at the institute. Aragão was originally interested in bacteriology, the topic of the doctoral dissertation he had defended in 1905: "Ensaios soroterápicos nas moléstias produzidas por germens não cultivados" (Serum therapy trials in sicknesses caused by non-cultivated germs). Aragão moved from intern to assistant on July 1 of that year; he later held the posts of chief of laboratory, chief of service, biologist with the credentials of a professorship and, from 1942 to 1947, director of the Oswaldo Cruz Institute.[15]

During the institute's early years, young researchers there discharged a range of duties. For instance, while conducting his first research in bacteriology and protozoology, Aragão also prepared sera and vaccines, studied spirochetes, and investigated ticks that were known or suspected of being disease transmitters, thus acquiring a panoramic view of various areas of biology, especially microbiology. In 1906, in conjunction with Henrique da Rocha Lima, he published the results of studies on the plague: "Nova técnica para o diagnóstico da peste" (New technique for diagnosing the plague). That same year, Aragão began to dedicate himself to the systematic study of ticks, a line of research that led to his development of a veterinary product that Manguinhos began manufacturing in 1907. This was a vaccine against spirillosis in chickens, an infectious disease caused by *Spirillum gallinarum*, a bacterium transmitted to caged chickens by ticks of the family Argasidae called *Argas*.[16] But it was his work on *H. columbae* that put him on the international stage.[17]

Inspired by Schaudinn's work on *T. noctuae* and by the ensuing controversies, Aragão tried to reproduce the German researcher's observations in *Haemoproteus* (Haemosporidia), using infected pigeons, and in the mosquito *C. fatigans* as a possible intermediary host, but he did not meet with success. His suspicions about the transmitter of *Haemoproteus*

then fell on Hippoboscidae (Diptera), a pigeon parasite found in large quantities in the bird's nest – a hypothesis raised by Adolpho Lutz. When Aragão began these investigations in 1906, the Sergent brothers published a paper in which they confirmed that *Lynchia* were the transmitters of *Haemoproteus* (Sergent, Sergent, 1906). Aragão then shifted his work to this Diptera, verifying the development of the parasite to the ookinete stage.[18] When he failed to trace it beyond this stage, he concluded that the parasite must complete its life cycle inside the pigeon's organism, where it was introduced through the bite of the insect.

One of the biggest question marks surrounding the life cycle of hematozoa, including that of malaria, was the disappearance of the parasite soon after its introduction into the vertebrate organism. Aragão successfully proved that the first part of the asexual cycle occurred within the pulmonary tissue of pigeons, where the parasite reproduced through schizogony[19], and that the invasion of erythrocytes only occurred during a second phase. Furthermore, he showed that there was no alternation in generation as Schaudinn had claimed; rather, it was common for the bird to be infected with both types of parasites, *Haemoproteus* and *Trypanosoma*. Thirteen to fourteen days after infection, Aragão found large meronts (schizonts, or multinucleate cells) with cytomeres[20] in the endothelial cells of the lungs of the infected bird. He also confirmed that the merozoites (daughter cells) produced by the meronts (mother cells) entered the erythrocytes and developed into gametocytes. As stated earlier, this was the first time that the existence of an exoerythrocytic cycle had been shown in a hematozoon.

In 1907, Aragão's findings were reported in a preliminary note in *Brazil-Medico*. The same year, his research was showcased at the Brazilian stand at the Hygiene Expo that was part of the 14th International Congress on Hygiene and Demography in Berlin, along with other scientific work produced at Manguinhos and information on Oswaldo Cruz's successful public health initiatives. The gold medal awarded at the event drew much attention in Brazil (Benchimol, 1990).

Aragão's work was well received and was published in 1908 in the prestigious *Archiv für Protistenkunde*, a periodical created by Schaudinn in 1902 and edited at that time by protozoologists Max Hartmann (1876-1962) and Stanilas von Prowazek (1875-1915). The *Revista Médica de São Paulo* published a summarized version of the article that same year (Aragão, 1908a, 1908b).

Aragão's discovery was one of the Manguinhos Serum Therapy Institute's great accomplishments in the area of protozoology; in 1908, thanks to these great successes, it was transformed into the Oswaldo Cruz Institute.[21] Despite the progress that had been made in protozoological, entomological, and helminthological research there, these disciplines were only officially institutionalized when the new institute was structured. The immediate consequences of the changes were that Adolpho Lutz became part of this research group, transferring in from the Bacteriological Institute in 1908, and that three important German researchers came for a six-month stay: chemist Gustav Giemsa (*1867-1948)* and Prowazek and Hartmann[22], the latter two mentioned earlier.

The German protozoologists arrived in Brazil at a momentous time in the history of the institute and of Brazilian science, that is, the era of Carlos Chagas's discovery of American trypanosomiasis. Prowazek and Hartmann were to wield great theoretical

influence in Chagas's studies and in future research in the field of protozoology, a discipline that became a definitive priority at the institute (Sá, 2005). During his six months at Manguinhos, Prowazek worked directly with Aragão, especially conducting research on smallpox and *Chlamydomonas*. In 1909, Aragão accompanied the German protozoologist on his return trip to Germany and there undertook work at Hamburg's Institute for Maritime and Tropical Diseases (Institutes für Schiffs-und Tropenkrakheiten) and also at the Munich Zoological Institute. Aragão later visited the Pasteur Institute and the Villefranche-sur-mer Marine Station in France.

Some years later, in 1915 to be precise, Oswaldo Cruz had the following to say about Aragão during a speech on diseases caused by protozoans in Brazil, pronounced at the National Library:

> I cannot fail in my duty to call to mind with reverent admiration and in heartfelt tribute Aragão's research on *Haemoproteus* parasites of pigeons. Conducted here at a difficult time, when technique had not attained the refinements that facilitated later research, these studies are truly remarkable, and it was this research that undoubtedly called the scientific world's attention to Brazilian work in protozoology. Relying on his own efforts and working alone, Aragão successfully and brilliantly traced the life cycle of *Haemoproteus* parasites of pigeons, definitively proving their transmission by the *Lynchia* flies of which it is a parasite. This study, amply confirmed by all who later turned to the topic, was the starting point for elucidation of other, correlated questions and represents one of the finest achievements in modern protozoology (Cruz, 1915, p.345-346, 353-356, 1972, p.738).

As stated earlier, Aragão clarified the matter of the disappearance of sporozoites following their introduction into birds by mosquitoes. His findings were confirmed years later but only for parasites that do not infect humans, that is, Haemoproteidae (family of protozoans that includes the genera *Haemoproteus* and *Leucocytozoon*).[23] In the case of the family Plasmodiidae, which includes the parasites that cause human malaria, the exoerythrocytic cycle was only solved thirty years after Aragão's work. This delay was in part due to the belief induced by Fritz Schaudinn that sporozoites head straight to erythrocytes when inoculated by mosquitoes, initiating their schizogonic cycle there. In a paper published in 1902, Schaudinn provided a detailed description of the path of the sporozoites of *Plasmodium vivax* (the cause of benign tertian malaria in humans) following inoculation by invertebrates. In his illustrations, he showed the successive aspects of the sporozoite's penetration of the erythrocyte and its ensuing development until it occupies one-quarter of the diameter of the cell and reaches the middle schizont stage (Paraense, 1944).

Although all attempts to prove Schaudinn's theory failed, he was considered such an authority in the matter that his life cycle of Plasmodiidae was deemed precise, even though there were no visible signs of the parasites after the insect had introduced them into the vertebrate organism and even though they appeared in the blood only ten days after infection.[24] In his book on protozoology, published in 1926, British parasitologist Charles Wenyon (1878-1948) made the following observations in comparing information on Haemoproteidae and Plasmodiidae: "In the Plasmodiidae ... the sporozoite ingested by the invertebrate, instead of entering the endothelial cells of the vessels to initiate the schizogony cycle, directly invade the red blood-corpuscles themselves, in which the whole

schizogony cycle terminating in the production of gametocytes takes place" (Wenyon, cited by Paraense, 1955, p.419).

Schaudinn's theory held for several decades and was also adopted in a number of teaching books, such as *Précis de parasitologie*, by Émile Brumpt (1877-1951), where we read: "All their stages of development in the vertebrate are found in the red blood cells" (Brumpt, 1936b, cited by Paraense, 2004, p.439). Similar interpretations can likewise be found in works of purely zoological interest. In 1940, Lybbie Henrietta Hyman (1888-1969), zoologist with the American Museum of Natural History in New York, published the first of a series of six books on invertebrates, from Protozoa to Ctenophora. In the order Haemosporidia, the author described the group's life cycle in accordance with what Schaudinn had postulated in 1902: "The type genus is *Plasmodium* (including *Laverania* and *Proteosoma*), causative organism of human and bird malaria ... *The sporozoites penetrate red blood corpuscles* where they grow as amoeboid organisms [in vertebrates]" (Hyman, 1940, p.153; emphasis added).

The reigning consensus among malariologists and protozoologists continued to be that "their cycles of development are completely known, though certain details of this development and many of the factors which regulate it have yet to be elucidated" (Wenyon, cited by Paraense, 1955, p.419).

The demise of Schaudinn's theory: the discovery of *Plasmodium gallinaceum* and the Brazilian contribution

The use of bird hematozoa became a solid model in researching malaria following Ronald Ross's late nineteenth-century discovery. In the first half of the twentieth century, it became the standard method for elucidating the life cycle of human malaria and for developing new medications.

Plasmodium relictum, the same parasite used in Ross's research, became the main model for chemotherapeutic studies following the 1911 publication of the work of Greek physician Phokion Kopanaris. Then at Hamburg's Institute for Maritime and Tropical Diseases, Kopanaris (1911) used *P. relictum* – a species to which various bird types were susceptible – to infect canaries in order to test the effects of quinine and Salvarsan. Although the latter drug was used to fight syphilis, it had proven to have some effect on human malaria parasites. Kopanaris managed to show that drugs used to treat human malaria were also efficacious in treating the avian version (Slater, 2009, p.45). In 1914, Kopanaris's conclusions were corroborated in experiments conducted at the Georg-Speyer-Haus Institute in Frankfurt, headed by Ehrlich. There it was shown that, with the exception of arsenics, all compounds efficacious in treating human malaria had an impact on avian malaria (Dünschede, 1971, p.63).

In the 1920s, these discoveries prompted physician Wilhelm Roehl (1881-1929) – who had left the institute headed by Erlich and was director of the chemotherapy laboratory at Bayer pharmaceutical in Elberfeld – to adapt the Kopanaris model to large-scale experiments (Dünschede, 1971, p.62). Using a logic inverse to the Greek doctor's, Roehl wanted to discover a substance active against avian malaria that could be proven efficacious against

human malaria (Slater, 2009, p.46). The big challenge was to arrive at a controlled method for administering the drugs to birds. Most researchers simply injected the substances diluted in water, which on the one hand provoked toxic side effects and on the other limited possible doses to very small quantities. At the Frankfurt institute, test substances were administered in ration but this method had serious flaws, especially because a large quantity of the substance was needed and it was uncertain how much of the dose was ingested by each bird. Roehl tried giving the drug substance to animals by attaching a thin urethral catheter to a syringe, allowing for measurement of the exact quantity of the dose to be administered. Roehl's method paved the way for controlled drug experimentation with birds (Dünschede, 1971).

One of the goals of the research in Elberfeld was to refine the toxic parts of the quinine molecule. Although the substance was efficacious in eliminating schizonts, it had countless side effects, like vomiting, hypertension, arrhythmia, and vision problems, in addition to its bitter taste and the fact that constant relapses were noted following treatment. In the mid-1920s, chemotherapists and chemists from Elberfeld, working together on manipulation of the quinine molecule, discovered the first synthetic drug against malaria, which they dubbed plasmoquine. Roehl tested the new compound on canaries infected with *P. relictum*. In addition to being well tolerated, it was considered thirty times more active than quinine and other compounds in the series developed by Elberfeld researchers (Dünschede, 1971, p.67; Slater, 2009, p.64). Roehl was able to observe that sexual forms were destroyed faster than schizonts, contrary to the effect of quinine. After a short time in laboratory, plasmoquine was tested on humans. Franz E. Sioli, director of the regional asylum in Düsseldorf, tested it on forty patients with *paretic neurosyphilis* infected with benign tertian malaria[25], while Peter Mühlens of Hamburg's Institute for Maritime and Tropical Diseases tested it on 134 outpatients. Since the latter represented either chronic cases or recent relapses (already having been treated with quinine in most cases), Mühlens suggested that the factory move to wide-scale testing of the product on all possible cases of the disease in the world's malaria hot spots. Mühlens was personally in charge of testing in the Balkan region of Bulgaria, Greece, and Yugoslavia, which he knew well from wartime. In 1926-1927, he also visited Central and South America, where he conducted experiments and gathered information on the use of plasmoquine in Venezuela, Costa Rica, Guatemala, Colombia, Panama, San Salvador, and Mexico. Otto Fischer, also from the Hamburg institute, tested the drug in Sierra Leone, Africa, while Roehl and Werner Schulemann, from Bayer, did so in Spain and Italy (Dünschede, 1971; Wulf, 1994; Slater, 2009). These experiments showed that plasmoquine's effect on tertian and quartan malaria was comparable to quinine's, but the effect of plasmoquine against schizonts (the multinucleate cells produced during asexual division inside parasited cells) rarely surpassed that of quinine in the tropics, while it was shown to act against gametes.[26] This was the first time chemotherapeutic experiments on laboratory birds had been successfully used for the benefit of humans. However, plasmoquine, like quinine, displayed high rates of toxicity, like cyanosis and biochemical disorders (decreased ability for the blood to carry oxygen). Roehl passed away in 1929 before finalizing laboratory tests of the drug and before establishing a standard method for ascertaining qualitative differences between

plasmoquine and quinine. His successor, Walter Kikuth (1896-1968), took over conducting the experiments.

Kikuth, then a researcher with the Institute for Maritime and Tropical Diseases in Hamburg, was invited to head up the Chemotherapy Section at Bayer by the company's scientific director, Philipp Heinrich Hörlein (1882-1954). In a letter dated June 18, 1929, Hörlein explained why he had decided to ask a member of the Hamburg institute to join Bayer: "On my last visit to Hamburg, I asked Professor Mayer where they [Mayer and Nocht] would stand, should I offer you the post here of the late Dr. Roehl ... It occurred to me that [this] ... would further strengthen the scientific relations and ties of friendship developed in recent years between the Tropical Disease Institute in Hamburg and the scientific section in Elberfeld" (Dünschede, 1971, p.139).

Kikuth had joined the institute in November 1924, after working for nearly two years at the University of Hamburg as an assistant at the Institute of Pathology and at the school's clinic. At the Institute for Maritime and Tropical Diseases, he served as scientific assistant at Martin Mayer's laboratory, conducting research on Oroya fever, bartonellosis, and malaria. Kikuth left the institute on October 1, 1929, to head Bayer's chemotherapy laboratory in Elberfeld. There he began a productive new phase in studies of avian malaria and engaged in prolific scientific exchange with foreign researchers, as we will see later.

While still an assistant at the Hamburg institute, Kikuth had spent six months in Brazil, from October 1927 through April 1928, working at the Oswaldo Cruz Institute's protozoology laboratory, with Henrique Aragão then at its helm. He learned of Aragão's work with Haemoproteidae while there. During this period, Kikuth established a partnership with Paul Regendanz (1888-1958), a German physician living in the nearby town of Petropolis who was a collaborator at the Oswaldo Cruz Institute's protozoology laboratory. Together they studied vertebrate hemoparasites, especially *Babesia* and their life cycle in ticks. This research was of interest to Henrique Aragão since he had devoted himself to ticks and protozoans from the outset of his career, as we saw earlier. The two German researchers worked with marsupials (the opossums *Didelphis marsupialis* and *Metachirus nudicaudatus*) and made a valuable contribution to our understanding of haemocytozoon fauna in New World species. They found two new species of mouse opossums, which they described as *Nuttalia brasiliensis* and *Haemogregarina metachiri* (Regendanz, Kikuth, 1928a, 1928b).

Following this rewarding partnership, Kikuth went back to Germany in April 1928, while Regendanz continued working on *Babesia* and ticks for some time at Eduard Reichenow's laboratory at the Institute for Maritime and Tropical Diseases. Upon returning to Brazil, he continued conducting investigations with researchers at the Oswaldo Cruz Institute's protozoology laboratory (Regendanz, Muniz, 1936).

When he took over Bayer's chemotherapy laboratory, Kikuth resumed the experiments with avian malaria begun by Roehl, using not only *Plasmodium* (*P. relictum*) but also *Haemoproteus* parasites as models. Roehl's methodology failed to be adequate in proving plasmoquine's feature of interest, that is, its effect on gametes. When infected with *Haemoproteus*, schizogony (cell division) occurs in the endothelial cells of the internal organs, as first described by Henrique Aragão, and the sexual forms corresponding to the

gametes of tropical malaria are found in the peripheral blood. *Haemoproteus* parasites do not undergo erthrocytic schizogony (cell division inside of erythrocytes) like *Plasmodium* but instead transform directly into gametocytes. The absence of this process within red blood cells is the only way to distinguish between Haemoproteidae and Plasmodiidae. This meant plasmoquine could be tested on *Haemoproteus* parasites, since the substance had a proven gametocidic effect and would make it possible to ascertain the effect of plasmoquine and new chemical substances on gametes, both qualitatively and quantitatively.[27]

Kikuth's experiments used *Padda oryzivora* birds (*tentilhão dos arrozais*), naturally infected with *Haemoproteus oryzivorae*.[28] He proved that plasmoquine had the same influence on gametes of *Haemoproteus* and of tropical malaria, unlike quinine, which did not affect the gametes of both.

Again using *Plasmodium* and *Haemoproteus* parasites, Kikuth began testing new substances that might have an effect on schizonts (Kikuth, 16 out. 1930). In 1930, he successfully tested a new compound on birds, developed in Elberfeld by chemists Hans Mauss (1901-1953) and Fritz Mietzsch (1896-1958). Called Atebrine, it was the first synthetic compound that rivaled quinine in eliminating schizonts. As with plasmoquine, Atebrine was clinically tested by Sioli on paretic patients and by Mühlens at the Hamburg institute and in Central and South America. Atebrine was put on the market in 1932.

In mid-1932, the Dutch-Indian monopoly over quinine was threatened by new treatment discoveries, and exports of *P. oryzivora* birds were banned. Experimental work with *Haemoproteus* was hampered by Bayer's inability to purchase these birds. In hopes of not interrupting research, I.G. Farben's representative[29] in Delhi, Doctor Urchs, was contacted about exporting birds from India (Dünschede, 1971, p.76). These problems were overcome three years later, when a new bird *Plasmodium* was discovered and then became broadly used as a model in chemotherapeutic experiments. These studies led at long last to the uncovering of the exoerythrocytic cycle in Plasmodiidae, invalidating Schaudinn's erroneous theory, which had been embraced by malariologists for over three decades. Let us examine the circumstances under which this occurred.

In 1935, French parasitologist Émile Brumpt described a new *Plasmodium* (*P. gallinaceum*) using a smear of *G. gallus*, a species originating in Eastern Asia, India, and Sri Lanka which later gave rise to today's domestic hen. A student of Brumpt's had brought the smear from Vietnam in 1910 and it had remained in storage in his laboratory for quite some time. During a trip to the Far East in 1935-1936, the French scientist had an opportunity to bring strains of the parasite to Paris. He then conducted experiments to verify its receptivity in different birds and proved transmission by the mosquito *Aedes aegypti* (Brumpt, 1935, 1936a). Brumpt pointed out the advantages of using this new parasite in chemotherapeutic studies and distributed strains to different laboratories and research institutes.

Several advantages of using this particular *Plasmodium* were touted: its size, how easy it was to find a host (it was experimentally possible to infect domestic hens, geese, pheasants, quails, and peacocks), and the fact that it could be transmitted by some species of mosquito (Slater, 2009, p.53; 2005, p.281). The availability of an experimental model that could be easily managed bolstered research on malaria in diverse parts of the world.

In 1936, when he learned that Henrique Aragão wanted to receive strains of the new *Plasmodium*, Brumpt expressed his concern about shipping the material to Brazil, since its transmission by *A. aegypti* had just been proven. In a letter to Aragão dated October 23, 1936, he explained:

> I had the pleasure of seeing your collaborator, Dr. Penido, at the Congress in London ... He spoke to me of your wish to obtain the *Plasmodium gallinaceum* chicken virus ... However, at the moment something new keeps me from sending you the virus, and here is the reason: I have just ascertained that the mosquito *Stegomyia fasciata* is highly receptive to this parasite, which develops in these insects in 100 percent of the cases. Since the natural disease is often fatal to chickens, whether by inoculation or by insect bite, I would have scruples about being the indirect cause of acclimatizing a similar parasite, which does not exist in your country (Brumpt, 23 out. 1936).

Despite this concern, Aragão obtained strains of *P. gallinaceum* and research was conducted at the Oswaldo Cruz Institute, leading to significant new contributions to our understanding of the exoerythrocytic cycle of the new parasite.

In 1937, Sydney James and Parr Tate, researchers at Cambridge, England, demonstrated the existence of an exoerythrocytic cycle in *P. gallinaceum*.

> This parasite, like other members of the family Plasmodiidae, has a schizogonic cycle of development in the circulating red blood corpuscles of its vertebrate host (the domestic fowl) and a sporogonic cycle of development in its insect host which, as Brumpt has shown, is the yellow fever mosquito *Stegomyia fasciata* (*Aedes aegypti*). But it appears from our work that it has, in addition, a hitherto unrecognized schizogonic cycle of development occurring in reticulo-endothelial cells of the spleen, liver, kidneys and other internal organs and particularly, in certain cases, in the reticulo-endothelial cells which line the capillaries of the brain" (James, Tate, 1937, p.545).

The work done by these scientists solved one of the most intriguing questions surrounding the cycle of the malaria parasite: the immediate destination of the sporozoite when placed in contact with the tissues of the vertebrate by the mosquito bite.[30] From then on, many studies were conducted both with *P. gallinaceum* and with other species of *Plasmodium*, confirming James and Tate's findings. Despite these positive results, many questions remained unanswered. Kikuth had been carrying out studies in chickens at Bayer using Brumpt's *P. gallinaceum* since 1936; he reported his results to Brumpt in these terms:

> We were indeed able to confirm James's findings in one of the chickens that you sent. We found the first parasites in the blood of the animal on the seventh day (May 10) after infection of the blood ... Based on this result, I think it is possible to infer that in terms of a special cycle of development, endothelial schizogony does not constitute a state that precedes the development cycle in the blood. In any case, we are working hard to move forward with our research in this direction and wish to thank you once again for providing us with this extremely interesting strain (Kikuth, 17 jun. 1937).

In Brazil, another young researcher from the Oswaldo Cruz Institute would make a valuable contribution to our understanding of the exoerythrocytic cycle of Plasmodiidae. In 1939, two years after completing medical school at the Recife Faculty of Medicine

(Faculdade de Medicina do Recife), Wladimir Lobato Paraense (1915-) joined the institute as a research assistant with the Service for the Study of Major Endemic Diseases (Serviço de Estudo das Grandes Endemias), where Evandro Chagas was director. The following year Lobato Paraense was appointed to give class on blood cells and the pathology of malaria as part of the malaria course that Carlos Chagas's son coordinated at Brazil's Northern Institute of Experimental Pathology (Instituto de Patologia Experimental do Norte), in Belém, Pará. During his preparation for this new work, Lobato Paraense learned about the parasitic cycle of *P. gallinaceum*, then only recently discovered by James and Tate, and also about the subsequent work of Giulio Raffaele (1938), Kikuth and Lilly Mudrow (1938), and others. Despite these investigations, some aspects of the exoerythrocytic cycle of the parasite were still unclear – for instance, the moment when exoerythrocytic forms appeared during the course of malarial infection. For some authors, once inoculated, the sporozoite immediately initiated exoerythrocytic schizogony. For others, the cycle only takes place after a blood infection has been established. Lobato Paraense wanted to understand the exact process and so he conducted a series of research studies using strains of *P. gallinaceum* previously acquired by Henrique Aragão and *A. aegypti* mosquitoes furnished by the Rockefeller Foundation's yellow fever laboratory, located on the campus of the Oswaldo Cruz Institute. Lobato Paraense successfully identified the exact moment when the exoerythrocytic forms appeared following inoculation into the tissue. His first findings were published in 1943: "around three days after inoculation," he wrote, "a relatively large quantity of elements resembling exoerythrocytic forms can be seen at the point of inoculation inside reticulo-endothelial cells of the subcutaneous tissue" (Paraense, 1943, p.356). Further, Lobato Paraense also confirmed that two types of schizogony occurred during the incubation period: one of schizonts with large nuclei and another of schizonts with much smaller nuclei.[31] Using the *P. praecox* model in canaries, and independent of Lobato Paraense, Edward Reichenow (1883-1960) and Lilly Mudrow (1908-1957), of the Institute for Maritime and Tropical Diseases, obtained identical results that same year. Like the Brazilian researcher, the Germans pointed to the morphological differences between the schizonts, which they called macroschizonts and microschizonts (Paraense, 1947, p.102). In 1944, Americans Clay G. Huff and Frederick Coulston published a conclusive paper on the destination of sporozoites of *Plasmodium* in birds following inoculation, supplementing the research by Paraense and by Reichenow and Mudrow.[32] As mentioned earlier, the Brazilian investigator's innovative research only attained international recognition following the end of World War II, by British parasitologist Charles M. Wenyon (1945).

In 1947, British researchers Henry Edward Shortt (1887-1987) and Percy Cyrill Claude Garnham (1901-1994) finally elucidated the exoerythrocytic cycle of human malaria. But it was only in 1980 that American and British researchers discovered hypnozoites, which are the latent forms of the exoerythrocytic cycle (or hepatic cycle) – at long last deciphering the riddle of relapsing human malaria (Krotoski et al., 1980).

In terms of scientific discoveries and the circulation of knowledge, Brazilian researchers made substantial contributions to our understanding of the life cycle of malaria parasites of humans and other vertebrates, their insect vectors, and disease prevention.[33] Despite significant advances in malaria research during the twentieth century, the malady still

ranks as the world's number one endemic disease, and mortality rates remain high. Birds have been replaced with simians and mice as experimental models and new challenges are constantly being posed, especially in the search for anti-malarial and anti-relapse drugs using natural products.

NOTES

[1] In this and other citations of texts from non-English languages, a free translation has been provided.

[2] The first Haemosporidia were discovered by British scientist Ray Lankester, who in 1871 described *Drepanidium ranarum*, an amphibian hemoparasite.

[3] In 1884, Laveran showed Ettore Marchiafava and Angelo Celli the new parasite he had discovered in 1880, which he believed to be the causative agent of human malaria. The two Italian researchers, who had a powerful microscope with oil immersion lenses, confirmed Laveran's theory in that this was an animal parasite, which they called *Plasmodium*. In 1885-1886, Camillo Golgi (1843-1926) showed the relation between cyclical development of the parasite and the periodicity of fevers; he deduced that the tertian and quartan fevers were caused by distinct species of *Plasmodium* (*P. vivax* and *P. malariae*). Marchiafava, Celli, Amico Bignami (1862-1929), Giuseppi Bastianelli (1862-1959), and others demonstrated that malignant tertian fever was caused by a different parasite; it was named *Haematozoon falciparum* in 1897 by U.S. parasitologist William H. Welch and later included in the genus *Plasmodium*.

[4] Haemosporidia are microscopic, intracellular protozoan and sporozoan parasites found in the bloodstream and tissues of reptiles, mammals, amphibians, and birds; their vectors are blood-sucking Diptera. They belong to the kingdom Protista, phylum Apicomplexa, order Haemosporidia.

[5] These species were included in the genus *Plasmodium*, established by Marchiafava and Celli in 1885. The genus *Haemamoeba* became a sub-genus of the genus *Plasmodium*, which now encompasses species that affect birds.

[6] In 1912, physicians Charles C. Bass and Foster M. Johns, with the laboratory for Tropical Medicine and Hygiene at Tulane University in New Orleans, released an article announcing the successful *in vitro* cultivation of *P. vivax* and *P. falciparum*. But these initial results were limited to a single cycle of the parasite and cultivation time to just a few days (Bass, Johns, 1912). In the following decades, efforts were made to lengthen the life and expand the cycles of these parasites *in vitro*, as well as avian and human malaria parasites. It was only in 1976 that a continuous culture of *P. falciparum* was achieved (Jensen, 2002).

[7] See Desowitz (1991, p.172). Variations on Romanowsky's method were undertaken by German chemist Gustav Giemsa, in 1902, and by U.S. pathologist James Homer Wright around the same time.

[8] The taxonomic classification of Protista remains controversial today. As new methodologies and techniques have developed out of molecular biology, immunology, biochemistry, and genetics, new taxonomic classifications have been proposed (see Adl et al., 2007).

[9] Coccidia are single-celled parasites belonging to the phylum Apicomplexa, class Conoidasida.

[10] For a detailed study on the discoveries of Ross and the Italian researchers, see Fantini, 1999, and Bynum, 1999.

[11] In 1905, Fritz Schaudinn, along with Erich Hoffman, discovered the bacterium *Spirochaeta pallida* (also known as *Treponema pallidum*), the causative agent of syphilis.

[12]Agar is a hydrocolloid extracted from different genera and species of red seaweed; it consists of a heterogeneous mixture of agarose and agaropectin. Novy and MacNeal developed a procedure for growing trypanosomes in a pure culture in the condensation water from slanted pipes of blood agar; they were the first to cultivate pathogenic protozoans. For more on this, see Long (1959, p.335).

[13] On the São Paulo Bacteriological Institute, see Lemos, 1954.

[14] See Lutz's studies on *Drepanididae* and pebrine in Benchimol, Sá, 2005 (p.821-840, 843-878).

[15] Aragão was born in the city of Niterói, Rio de Janeiro, on Dec. 21, 1879. His parents were Doctor Francisco Pires de Carvalho Aragão and Elisa de Beaurepaire-Rohan Aragão and his maternal grandfather was the Viscount of Beaurepaire-Rohan, founder of Brazilian cartography. For bibliographic information on the researcher, see Nery-Guimarães, 1955.

[16] For a history of the Oswaldo Cruz Institute, see Stepan, 1976, and Benchimol, 1990.

[17] Infection by *Haemoproteus* is known as pseudo-malaria, owing to similarities with species of *Plasmodium*.

[18] Years later, Helen Adie achieved further progress, showing that parasites reached the sporozoite phase inside the mosquito, as did malaria parasites, introduced into the definitive host through the insect's salivary glands (Adie, 1915).

[19] Schizogony is the process by which a cell divides into three or more cells; it is common in protozoans of the genus *Plasmodium*.

[20] Cytomeres are structures that are formed when the contents of a large, simple schizont or meront (multinucleate cell) divide into multiple daughter cells (merozoites) during schizogony. They are found in some species of sporozoans during the process of asexual exoerythrocytic division.

[21] This discovery was surpassed only two years later, with Carlos Chagas's masterful discovery of American trypanosomiasis in 1909. For more on this, see Kropf, 2009.

[22] Giemsa and Prowazek were staff researchers at the Institute for Maritime and Tropical Diseases **in** Hamburg, founded around the same time as the Brazilian institution. Hartmann was with the Infectious Disease Institute, in Berlin. When he invited Prowazek, meant for him to help organize a protozoology laboratory at Manguinhos (Livro..., n.d., p.24).

[23] Despite Aragão's evidence of an exoerythrocytic phase inside tissue cells and also of Novy and MacNeal's work contesting the notion of alteration of generation, many researchers persisted in agreeing with Schaudinn, who enjoyed great prestige among scientists. In 1913, Brumpt had this to say in his book *Précis de parasitologie*: "according to Schaudinn's work, the life cycle [of parasites] is quite complex and when they reach the mosquito's midgut, they become trypanosomes. Therefore, there is a hitherto unsuspected kinship between flagellates and sporozoans" (Brumpt, 1913, p.93).

[24] In 1901, before the publication of Schaudinn's work, Grassi had suggested that sporozoites inoculated into the human body must first pass through a stage of development outside the red blood cells, producing infecting elements capable of entering the erythrocytes. But when Schaudinn's work was released, Grassi was one of the first to wholeheartedly support the German scientist's theory.

[25] The malariotherapy method was devised in 1910 by Austrian physician Julius Wagner von Jauregg on syphilitic patients presenting neuropsychiatric disorders and was used mainly to treat general paralysis caused by syphilis. Brazilian psychiatric hospitals began applying it in the 1920s (Lopes, 2001).

[26] In cases of relapsing tropical malaria caused by *P. falciparum*, quinine began to be administered together with plasmoquine, with better results.

[27] The *Plasmodium* cycle begins when the *Anopheles* mosquito inoculates sporozoites directly into the bloodstream. They then travel to the liver and transform into cryptozoites. At the end of the growth cycle, the cryptozoite nucleus begins asexual reproduction by dividing multiple times. This produces a multinucleate form called the schizont. The schizont ruptures, releasing merozoites. Known as pre-erythrocytic schizogony, this entire stage takes six to sixteen days post-inoculation. Each merozoite released during the prior phase infects an erythrocyte. Inside the erythrocyte, the merozoite then undergoes schizogony and develops into a trophozoite. The trophozoite nucleus starts undergoing asexual reproduction, producing a schizont, which ruptures and releases merozoites that can then repeat the asexual process or begin the sexual cycle. The repetition of the asexual cycle inside the erythrocytes is known as the erythrocytic cycle. In this case, the periodicity with which schizogony (asexual division) is repeated depends upon the species and is associated with the pace of bouts of fever.

In the case of the sexual cycle, male or female gametocytes are produced from merozoites. The gametocytes formed in humans are ingested by an *Anopheles* mosquito during bloodsucking. Fertilization occurs in the mosquito's midgut. Gametocytes fuse to form oocytes inside cells of the mosquito's intestinal epithelium. Sporulation causes the oocyte to rupture inside the mosquito's hemocoel, releasing the sporozoites. These travel to the mosquito's salivary gland and are later inoculated into the vertebrate host.

[28] A good share of these birds (*Padda oryzivora*), which come from the former colonial regions of the British Indies and Dutch colonial territories, suffer from a chronic infection of the erythrocytes by *H. oryzivorae*, transmitted by Hippoboscidae.

[29] I.G. Farben (Interessen-Gemeinschaft Farbenindustrie A.G.) is a conglomerate of German chemical companies created in 1925.

[30] In 1931, while using malariotherapy to treat patients, James noted that relapses were common in those bitten by mosquitoes rather than inoculated with contaminated blood and that quinine was more efficient in cases of inoculation. He then formulated the hypothesis that instead of invading erythrocytes, sporozoites invaded the cells of connective tissue, a hypothesis that would be confirmed some years later (Paraense, 1944, p.474).

[31] Lobato Paraense published a paper on the exoerythrocytic cycle of malaria parasites in 1944 and on the pathogenic action of the exoerythrocytic forms of *P. gallinaceum* in 1946 (Paraense, 1944, 1946). See also Pays, 2010.

[32] These discoveries prompted important research into relapses, as Kikuth mentioned in his annual report for 1943: "With our new understandings of the process of the development of sporozoites in the vertebrate host, which in our view apply to human malaria, we can now provide a simpler explanation for a series of questions on the epidemiology and clinical presentation of malaria, whose interpretation had previously been so difficult ... Spring relapses are apparently caused by the persistence of reticulo-endothelial forms in the internal organs that survive the early phase of infection and, after some nine months of latency, make their clinical appearance by attacking the erythrocytes" (Kikuth, Feb. 29, 1944).

[33] See also Benchimol, Sá, 2005, 2006, and Benchimol, Silva, 2008.

REFERENCES

ADIE, Helen A.
The sporogony of Haemoproteus columbae. *Indian Journal of Medical Research*, New Delhi, n.2, p.671-680. 1915.

ADL, M. Sina et al.
Diversity, nomenclature, and taxonomy of protists. *Systematic Biology*, Oxford, v.56, n.4, p.684-689. 2007.

ARAGÃO, Henrique de Beaurepaire.
Über den Entwicklungsgang und die Übertragung von Haemoproteus columbae. *Archiv für Protistenkunde*, Jena, v.12, p.154-167. 1908a.

ARAGÃO, Henrique de Beaurepaire.
Sobre o cyclo evolutivo e a transmissão do Haemoproteus columbae. *Revista Médica de São Paulo*, São Paulo, v.11, n.20. 1908b.

ARAGÃO, Henrique de Beaurepaire.
Sobre o cyclo evolutivo do halterídeo do pombo: nota preliminar. *Brazil-Medico*, Rio de Janeiro, v.21, n.15, p.141-142, 1907.

BASS, Charles C.; JOHNS, Foster M.
The cultivation of malarial plasmodia (Plasmodium vivax and P. falciparum) in vitro. *Journal of Experimental Medicine*, New York, v.16, n.4, p.567-579. 1912.

BENCHIMOL, Jaime L.
Manguinhos do sonho à vida: a ciência na Belle Époque. Rio de Janeiro: Casa de Oswaldo Cruz/ Fiocruz. 1990.

BENCHIMOL, Jaime L.; SÁ, Magali R.
Apresentação histórica. In: Benchimol, Jaime L.; Sá, Magali R. (Ed.). *Adolpho Lutz*: obra completa. Adolpho Lutz e a entomologia médica no Brasil. v.2, livro 3, p.11-223. Rio de Janeiro: Editora Fiocruz. 2006.

BENCHIMOL, Jaime L.; SÁ, Magali R.
Insetos, humanos e doenças: Adolpho Lutz e a medicina tropical. In: Benchimol, Jaime L.; Sá, Magali R. (Ed.). *Adolpho Lutz*: obra completa. Febre amarela, malária e protozoologia. v.2, livro 1, p.43-244. Rio de Janeiro: Editora Fiocruz. 2005.

BENCHIMOL, Jaime L.; SILVA, André Felipe Cândido da.
Ferrovias, doenças e medicina tropical no Brasil da Primeira República. *História, Ciências, Saúde – Manguinhos*, Rio de Janeiro, v.15, n.3, p.719-762. 2008.

BRUMPT, Émile.
Carta a Henrique Aragão. Paris. Brumpt, E. Correspondance thématique, BPT.B10. (Archives de l'Institut Pasteur). 23 out. 1936.

BRUMPT, Émile.
Réceptivité de divers oiseaux domestiques et sauvages au parasite (Plasmodium gallinaceum) du paludisme de la poule domestique. Transmission de cet hématozoaire par le moustique Stegomyia fasciata. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*, Paris, n.203, p.750-752. 1936a.

BRUMPT, Émile.
Précis de parasitologie. 5ème. ed. Paris: Masson. 1936b.

BRUMPT, Émile.
Paludisme aviaire: Plasmodium gallinaceum n. sp. de la poule domestique. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*, Paris, n.200, p.783-785. 1935.

BRUMPT, Émile.
Précis de parasitologie. 2ème. ed. Paris: Masson. 1913.

BYNUM, William F.
Ronald Ross and the malaria-mosquito cycle. *Parassitologia*, Roma, v.41, p.49-52. 1999.

COX, Francis.
History of the discovery of the malaria parasites and their vectors. *Parasites & Vectors*, v.3, n.5. 2010. Disponível em: http://www.parasitesandvectors.com/content/3/1/5. Acesso em: 2 jul. 2010.

CRUZ, Oswaldo.
Oswaldo Gonçalves Cruz: Opera Omnia. Org., Emília Bustamante. Rio de Janeiro: Impressora Brasileira. p.721-738. 1972.

CRUZ, Oswaldo.
Algumas moléstias produzidas por protozoários: conferência feita na Biblioteca Nacional. *Brazil–Medico*, Rio de Janeiro, v.29, n.44, p.345-346; n.45, p.353-356. 1915.

CRUZ, Oswaldo.
Carta a Adolpho Lutz. Rio de Janeiro. Fundo Adolpho Lutz, pasta 213. (Museu Nacional do Rio de Janeiro). 15 abr. 1907.

DANILEWSKY, Basil.
Zur Parasitologie des Blutes. *Biologisches Zentralblatt*, Leipzig, n.5, p.529-537. 1886.

DESOWITZ, Robert S.
The malaria capers. New York: W.W. Norton. 1991.

DÜNSCHEDE, Horst-Bernd.
Tropenmedizinische Forschung bei Bayer. Düsseldorf: Michael Triltsch. 1971.

FANTINI, Bernardino.
The concept of specificity and the italian contribution to the discovery of the malaria transmission cycle. *Parassitologia*, Roma, v.41, p.39-47. 1999.

HUFF, Clay G.; COULSTON, Frederick.
The development of Plasmodium gallinaceum from sporozoite to erythrocytic trophozoite. *Journal of Infectious Diseases*, Arlington, n.75, p.231-249. 1944.

HYMAN, Libby Henrietta.
The invertebrates: protozoa through ctenophora. New York: McGraw-Hill. 1940.

JAMES Sydney P.; TATE, Parr.
New knowledge of the life-cycle of malaria parasites. *Nature*, London, n.139, p.545. 1937.

JENSEN, James B.
In vitro culture of Plasmodium. In: Doolan, Denise L. *Methods in molecular medicine*. Totowa: Humana Press. p.477-488. (Malaria Methods and Protocols, v.72). 2002.

KIKUTH, Walter.
Jahresbericht 1943. Chemotherapeutisches Laboratorium de I.G. Farben, Werk Wuppertal-Elberfeld. Bayer Business Services. Elberfeld, Deutschland. (Corporate History and Archives). 29. Feb. 1944.

KIKUTH, Walter.
[Carta de Walter Kikuth para Émile Brumpt]. Correspondência Brumpt, E. Laboratoire de Parasitologie para Direktion Werk Elberfeld II – 1929-1939. Bayer Business Services. Elberfeld, Alemanha. (Corporate History and Archives). 17. Jun. 1937.

KIKUTH, Walter.
Bericht über die Halteridium-Infektion des Reisfinken als Modellversuch. Bayer Business Services. Elberfeld, Deutschland. (Corporate History and Archives). 16. Okt. 1930.

KIKUTH, Walter ; MUDROW, Lilly.
Die endothelialen Stadien der Malariaparasiten in Experiment und Theorie. *Zentralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten*, Jena, n.142, p.114-132. 1938.

KOPANARIS, Phokion.
Die Wirkung von Chinin, Salvarsan und Atoxyl auf die Proteosoma- (Plasmodium praecox) Infektion des Kanarienvogels. *Archiv für Schiffs- und Tropenhygiene, Pathologie und Therapie exotischer Krankheiten*, Hamburg, v.15, p.586-596. 1911.

KRAUSE, Walther.
Über Blutparasiten. *Archiv für pathologische Anatomie und Physiologie, und für klinische Medizin*, Berlim, n.121, p.359-373. 1890.

KROPF, Simone.
Doença de Chagas, doença do Brasil: ciência, saúde e nação (1909-1962). Rio de Janeiro: Editora Fiocruz. 2009.

KROTOSKI, Wojciech A. et al.
Relapses in primate malaria: history of two populations of exoerythrocytic stages. Preliminary note. *British Medical Journal*, London, v.1, p.153-154. 1980.

LAVERAN, Alphonse.
Des hematozoaires des oiseaux voisins de l'hematozoaire du paludisme. *Comptes Rendus Hebdomadaires des Séances et Mémoires de la Société de Biologie et de ses Filiales*, Paris, v.43, p.127-132. 1891.

LEMOS, Fernando Cerqueira.
Contribuição à história do Instituto Bacteriológico (1892-1940). *Revista do Instituto Adolfo Lutz*, São Paulo, v.14, número especial, p.1-61. 1954.

LIVRO...
Livro de Assentamentos Funcionais. Rio de Janeiro. Fundo Instituto Oswaldo Cruz, livro 1, p.24. (Casa de Oswaldo Cruz, Fundação Oswaldo Cruz). s.d.

LONG, Esmond R.
Frederick George Novy (1864-1957). Washington: National Academy of Sciences, p.326-350. (Biographical Memoirs of the National Academy of Sciences, n.33). 1959.

LOPES, Maria Helena Itaqui.
Pesquisa em hospitais psiquiátricos, Disponível em : http://www.ufrgs.br/bioetica/psiqpes.htm. Acesso em: 10 ago. 2010. 2001.

MACCALLUM, William George.
Notes on the pathological changes in the organ of birds infected with haemocytozoa. *Journal of Experimental Medicine*, New York, v.3, n.1, p.103-116. 1898.

MACCALLUM, William George.
On the flagellated form of the malarial parasite. *The Lancet*, London, v.150, n.3872, p.1240-1241. 1897.

NERY-GUIMARÃES, Felipe.
Notas bio-bibliográficas do Prof. Dr. Henrique de Beaurepaire Aragão, Diretor Emérito do Instituto Oswaldo Cruz. *Memórias do Instituto Oswaldo Cruz*, Rio de Janeiro, v.53, n.2, 3, 4, p.143-156. 1955.

PARAENSE, Wladimir Lobato.
A fragment of malaria history. *Memórias do Instituto Oswaldo Cruz*, Rio de Janeiro, v.99, n.4, p.439-442. 2004.

PARAENSE, Wladimir Lobato.
The discovery of the schizogony cycle in the pigeon's halteridium and its influence on the advancement of protozoology. *Memórias do Instituto Oswaldo Cruz*, Rio de Janeiro, v.53, n.2,3,4, p.416-420. 1955.

PARAENSE, Wladimir Lobato.
Estudos sobre o ciclo exoeritrocitário do Plasmodium gallinaceum. *Memórias do Instituto Oswaldo Cruz*, Rio de Janeiro , v.45, n.1, p.101-118. 1947.

PARAENSE, Wladimir Lobato.
Ação patogênica das formas exoeritrocíticas do Plasmodium gallinaceum. *Memórias do Instituto Oswaldo Cruz*, Rio de Janeiro, v.44, n.1, p.147-191. 1946.

PARAENSE, Wladimir Lobato.
O ciclo exoeritrocitário dos parasitos da malária. *Memórias do Instituto Oswaldo Cruz*, Rio de Janeiro, v.41, n.3, p.469-493. 1944.

PARAENSE, Wladimir Lobato.
Aspectos parasitários observados no local inoculado com esporozoitos de Plasmodium gallinaceum. *Memórias do Instituto Oswaldo Cruz*, Rio de Janeiro, v.38, n.3, p.353-359. 1943.

PAYS, Jean François.
Characterisation of the exoerythrocytic stage of the asexual cycle of the human plasmodia: a painstaking process. *Bulletin de la Société de Pathologie Exotique*, Paris, v.103, n.4, p.272-279. 2010.

RAFFAELE, Giulio.
La fase primaria dell'evoluzione monogonica dei parassiti malarici. *Rivista di Malariologia*, Rio de Janeiro, v.17, n.5, p.331-343. 1938.

REGENDANZ, Paul; KIKUTH, Walter.
Sur un parasite du sang des quica (Metachirus quica), Nuttallia brasiliensis n. sp. et influence de la rate sur les infections latentes du sang. *Comptes Rendus Hebdomadaires des Séances et Mémoires de la Société de Biologie et de ses Filiales*, Paris, n.98, p.1567-9. 1928a.

REGENDANZ, Paul; KIKUTH, Walter.
Sur les hémogrégarines du 'gambá', (Haemogregarina didelphydis), de la 'quica', (Haemogregarina metachirin. n.sp.) et sur l'haemogregarina ratti. *Comptes Rendus Hebdomadaires des Séances et Mémoires de la Société de Biologie et de ses Filiales*, Paris, n.98, p.1565-1567. 1928b.

REGENDANZ, Paul; MUNIZ, Julio.
O Rhipicephalus sanguineus como transmissor da piroplasmose canina no Brasil. *Memórias do Instituto Oswaldo Cruz*, Rio de Janeiro, v.31. n.1, p.81-84. 1936.

REICHENOW, Eduard; MUDROW, Lilly.
Der Entwicklungsgang von Plasmodium praecox im Vogelskörper. *Deutsche tropenmedizinische Zeitschrift*, Hamburg, v.47, p.289-299. 1943.

SÁ, Magali Romero.
The tropical medicine in Brazil: the discovery of Trypanosoma cruzi in the German School of Protozoology. *Parassitologia*, Roma, v.47, p.309-317. 2005.

SCHAUDINN, Fritz.
Generations- und Wirtswechsel bei Trypanossoma und Spirochäte. *Arbeiten aus dem Kaiserlichen Gesundheitsamte*, Berlin, v.20, n.3, p.387-439. 1904.

SCHAUDINN, Fritz.
Studien über krankheitserregende Protozoen II. Plasmodium vivax (Grassi & Feletti), der Erreger der Tertianfiebers beim Menschen. *Arbeiten aus dem Kaiserlichen Gesundheitsamte*, Berlin, v.19, p.169-250. 1902.

SCOTT, Harold H.
A history of tropical medicine. London: Edward Arnold. 2 v. 1942.

SERGENT, Edmond; SERGENT, Étienne.
Sur le second hôte de l'Haemoproteus (Halteridium) du pigeon. *Comptes Rendus Hebdomadaires des Séances et Mémoires de la Société de Biologie et de ses Filiales*, Paris, v.58, n.2, p.494-496. 1906.

SIMOND, Paul-Louis.
Recherches sur les formes de reproduction asporulée dans le genre Coccidium. Note présentée par E. Metchnikoff. *Comptes Rendus Hebdomadaires des Séances et Mémoires de la Société de Biologie et de ses Filiales*, Paris, v.49, p.425-428. 1897.

SLATER, Leo B.
War and disease: biomedical research on malaria in the twentieth century. New Brunswick: Rutgers University Press. 2009.

SLATER, Leo B.
Malaria birds: modeling infectious human disease in animals. *Bulletin of the History of Medicine*, Baltimore, v.79, p.261-294. 2005.

STEPAN, Nancy.
Gênese e evolução da ciência brasileira. Rio de Janeiro: Artenova. 1976.

VALKIUNAS, Gediminas.
Avian malaria parasites and other haemosporidia. Boca Raton: CRC Press. 2005.

WENYON, Charles M.
Tropical medicine in war and peace. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, London, v.39, p.177-194. 1945.

WENYON, Charles M.
Protozoology. London: Bailliere, Tindall and Cox. v.2. 1926.

WULF, Stefan.
Das Hamburger Tropeninstitut, 1919 bis 1945: auswärtige Kulturpolitik und Kolonialrevisionismus nach Versailles. Hamburg: Reimer. 1994.